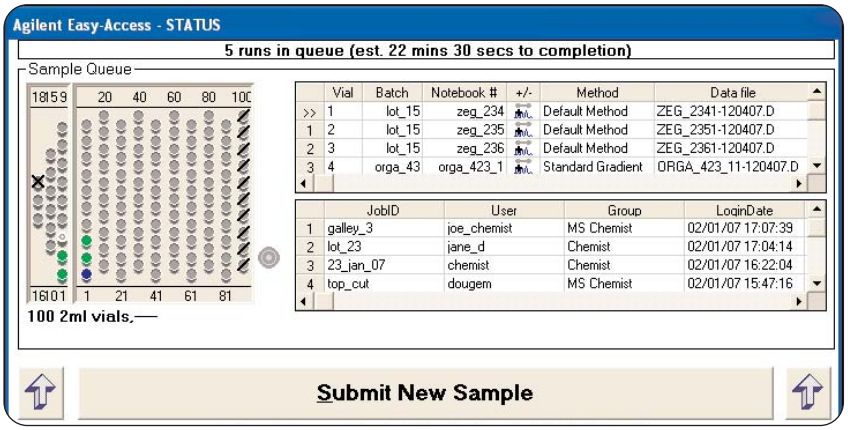
Continuous Operation of a High Throughput LC/MS System in a Walk-Up Environment

Application Note

Doug McIntyre


Agilent Easy-Access - STATUS
5 runs in queue (est. 22 mins 30 secs to completion)
Sample Queue
100 2ml vials
Vial Batch Notebook # +/- Method Data file
1 lot_15 zeg_234 Default Method ZEG_2341-120407.D
2 lot_15 zeg_235 Default Method ZEG_2351-120407.D
3 lot_15 zeg_236 Default Method ZEG_2361-120407.D
4 orga_43 orga_423_1 Standard Gradient ORGA_423_11-120407.D
JobID User Group LoginDate
galley_3 joe_chemist MS Chemist 02/01/07 17:07:39
lot_23 jane_d Chemist 02/01/07 17:04:14
23_jan_07 chemist Chemist 02/01/07 16:22:04
top_cut dougem MS Chemist 02/01/07 15:47:16
Submit New Sample

Abstract

In drug discovery, it is common for one LC/MS system to be shared by many medicinal chemists. The system should be capable of rapidly running large numbers of different samples without the need to pause the system. It should also be rugged enough so that it is difficult for an untrained chemist to put the system in a state that will either shut down the LC/MS or cause bad data to be produced for subsequent samples. This Application Note describes a system based on an Agilent 1200 Series Rapid Rapid Resolution LC system, configured with an autosampler capable of handling samples non-stop, along with Walk-Up software designed to take advantage of this capability.

Agilent Equipment

- 1200 Series Series Rapid Resolution LC
- 6140 Quadrupole LC/MS
- 1200 Series standard autosampler SL
- Easy Access software
- Data Browser software

Application Areas

- New drug library MW confirmation
- Purification fraction confirmation and assessment
- Lead optimization confirmation

Agilent Technologies

Introduction

In the development of new pharmaceutical products, there is a demand for shorter analysis times in order to analyze more samples per day on each LC/MS system. Previously, separation was sacrificed for throughput. Today, the use of 1.8 micron particle size columns on a rapid resolution LC/MS system allows to run a generic gradient that will perform adequate separation in most cases without requiring extended run times. This makes it ideal for walk-up applications where many chemists wish to run a broad range of samples on a single system. A previous publication[1] described such a system for rapid high throughput work. This publication describes how to use such a system with additional hardware and software in a walk-up environment designed for the continuous running of samples. It describes the components of an LC/MS quadrupole system optimized for such high speed chromatography and universal detection.

Experimental

The Agilent 1200 Series Rapid Resolution LC system described in the previous note[1] was modified by using a new Agilent 1200 Series standard autosampler SL (Agilent part number G1329B) fitted with the optional external tray (Agilent part number G1313-60004) and disposal tube (Agilent part number G1313-27302). The system consisted of the following modules:

- Agilent 1200 Series binary pump SL with micro degasser
- Agilent 1200 Series standard autosampler SL with thermostat.
- Agilent 1200 Series thermostatted column compartment with 10-port valve for column switching
- Agilent 1200 Series diode array detector (DAD) SL with the capability to acquire data with a sampling rate up to 80 Hz.
- Agilent 6140 quadrupole MS with scan speed up to 10,000 u/sec equipped with multimode source
- Column: ZORBAX SB C18, 3 x 50 mm, 1.8 µm particle size.
- Software: ChemStation Rev. B.02.01 SR1 with Easy Access Rev. 04.03

A close-up view of the sampler with external tray is shown in figure 1. The samples used included the Agilent Electrospray LC Demo Sample (Agilent part number 59987-20033) containing 4 sulfa drugs. In addition, a range of pharmaceutical compounds were provided by customers to check spectral quality.

Results and discussion

The Agilent 1200 Series standard automsampler SL allows for 600 bar operation making it suitable for use in an Agilent 1200 Series Rapid Resolution LC system. It can be fitted with an external tray that has 17 positions for samples and one drilled through position connected to a waste tube. When submitting the samples, the Easy Access soft-

Figure 1
Close up view showing the external tray mounted on the Agilent 1200 Series automsampler SL.

Conditions:	
Solvent A:	Water with 0.1 % acetic acid;
Solvent B:	methanol with 0.1 % acetic acid.
Flow:	2 mL/min for 3 mm column.
Gradient:	8 % B to 6 0 %B in 1 min.
Stop time:	1.5 min
Column compartment:	operated at 80 °C
DAD:	operated at 40 Hz, wavelength 272 nm/16, ref 360/100, spectra acquired across all peaks 210 – 400 nm
MS conditions:	
Multimode source:	in combined ESI-APCI mode, Scan range m/z 100 – 600,
Vaporizer:	200 °C
Dry Gas:	6 L/min at 265 °C
Nebulizer:	35 psi
Fragmentor:	150 V
VCap:	3000 V
Corona:	current 4 _A
Charging voltage:	2000 V

ware prompts the chemist to place them in the appropriate external positions after they have provided the necessary sample information and chosen an appropriate method. This can be seen in the software window shown in figure 2. The Easy Access software then directs the sampler arm to move the vials to the next available positions on the internal 100-vial tray. The software tracks all samples in the queue and directs the sampler to inject each sample with the appropriate method. On successful completion of the run and producing a report, Easy Access then directs the sampler arm to move the sample from the internal tray to the disposal position of the external tray. The vial drops through to a waste container. This discard feature can be delayed for several hours if desired. Easy Access can be configured to notify the submitter by email that the sample analysis is complete. This email can include attached raw data, reports or a data browser file.

This mode of operation allows the user to safely submit vials without reaching inside the sampler. In addition, the vial discard means the system can run continuously since the sampler tray never fills up. The Easy-Access software will even notify the system administrator by e-mail when the solvent reservoirs are running low or if any system error such as a leak has halted the system.

While the user can do a detailed review the results in the ChemStation Data Analysis or look at the printed reports, most prefer to do a simple review to determine the synthesis was done correctly with an appropriate yield. The Easy Access software will notify the chemist by email that the samples have been finished and include an attachment of the results for review. An example of the e-mail is shown in figure 3.

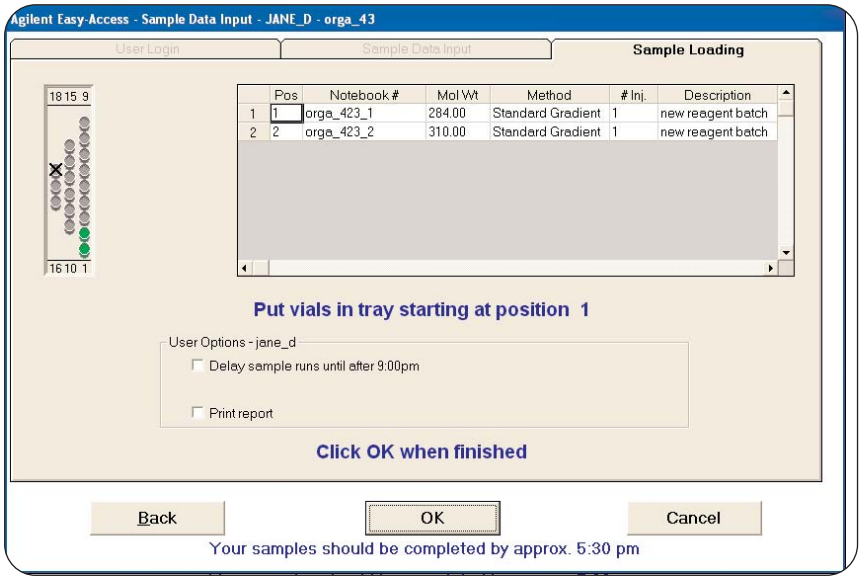

Agilent Easy-Access - Sample Data Input - JANE_D - orga_43
User Login
Sample Data Input
Sample Loading
18 15 9
16 10 1
Pos Notebook # Mol Wt Method # Inj. Description
1 1 orga_423_1 284.00 Standard Gradient 1 new reagent batch
2 2 orga_423_2 310.00 Standard Gradient 1 new reagent batch
Put vials in tray starting at position 1
User Options - jane_d
Delay sample runs until after 9:00pm
Print report
Click OK when finished
Back
OK
Cancel
Your samples should be completed by approx. 5:30 pm

Figure 2
Easy Access software prompting the chemist to place the samples in the appropriate locations. The bottom of the panel indicates the approximate time of completion.

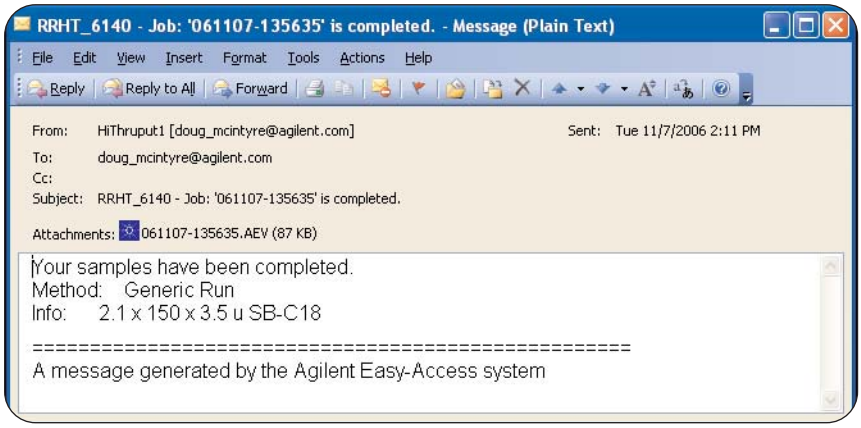

RRHT_6140 - Job: '061107-135635' is completed. - Message (Plain Text)
File Edit View Insert Format Tools Actions Help
Reply Reply to All Forward
From: HiThruput1 [doug_mcintyre@agilent.com]
Sent: Tue 11/7/2006 2:11 PM
To: doug_mcintyre@agilent.com
Cc:
Subject: RRHT_6140 - Job: '061107-135635' is completed.
Attachments: 061107-135635.AEV (87 KB)
Your samples have been completed.
Method: Generic Run
Info: 2.1 x 150 x 3.5 u SB-C18
==========
A message generated by the Agilent Easy-Access system

Figure 3
An example of an email notification provided by the Easy Access software.

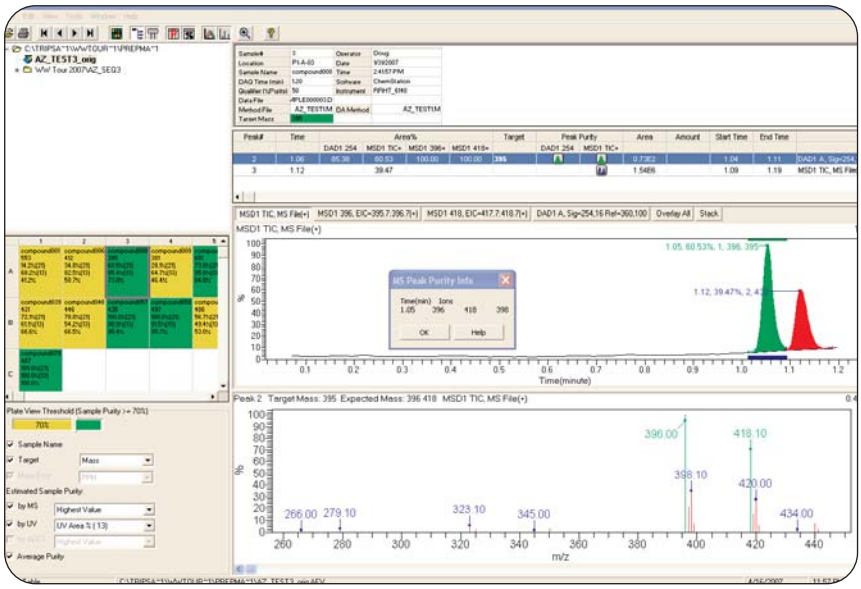


Figure 4
Data Browser results showing indicating the target compound was present along with an approximate purity.

The Data Browser software was designed to run at the chemist's desk to allow to quickly assess the data quality and provide easily customized reporting. Figure 4 shows an example of the Data Browser view. The chemist can quickly see sample purity, the spectrum of the target compound and any other major peaks as well as other key information.

Conclusion

The use of Rapid Resolution High Throughput columns allows for generic gradients providing good separation for a broad range of compounds while still keeping the run times short. This is ideal for high throughput systems used in a walk-up environment that is supporting many chemists per system.

Non-stop operation is possible by using the external tray option on the standard autosampler. This results in effectively unlimited sample capacity. The Easy Access software in combination with the Data Browser software allows the chemist to walk up to an instrument, submit samples and have the data sent to his desk when the samples are finished, improving productivity.

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www.agilent.com/chem/1200rr

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